

REMARKS

Applicant respectfully requests entry of the amendments and the following remarks into the record of the above-identified patent application. Claims 21, 22, 24-29, 31-42 and 47-49 are pending in this application upon entry of this paper. Applicant has amended claims 21, 22, 26, 34, 35, and 40 and has added new claims 47-49 to more clearly recite the claimed invention. No new matter has been added by any of the amendments.

Support for the new claims can be found in the specification as-filed as follows:

<u>Claim</u>	<u>Support in the Specification as-filed</u>
47	<i>See specification as-filed at pages 5-7.</i>
48	<i>See specification as-filed at page 7, lines 15-21.</i>
49	<i>See specification as-filed at page 4, lines 15-21.</i>

I. The Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 21, 22, 24-29, and 31-42 are rejected on pages 3-5 of the office action under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter, which applicant regards as the invention.

Claim 21 is rejected as allegedly vague and indefinite because it is unclear how the claimed method can differentially determine between 1) the presence of, and 2) the amount of, new synthesized target antibodies.

Applicant respectfully submits that claim 21 has been amended to overcome the rejection. Moreover, Applicant submits that the amendment to claim 21 is not a narrowing amendment, Applicant has amended claim 21 to recite, *inter alia*, "determining the presence of newly synthesized target antibody in a body fluid sample in response to an immunogen."

Applicant respectfully submits that the method encompassed by the pending claims can be used to detect the presence of antibody and/or also to detect the amount of

antibody. The preamble of claim 21 prior to the amendments made herein recited, *inter alia*, “[a] method of determining the presence or amount of newly synthesized target antibody in a body fluid sample in response to an immunogen.” (Emphasis added). One of ordinary skill in the art, when carrying out the assay as claimed, can choose which of the two options to carry out. However, to clarify the scope of the claims, Applicant has amended claim 21 to recite determining the presence of newly synthesized target antibody . . . Applicant has also added new claim 47 to encompass the method of determining the amount of a newly synthesized target antibody.

Applicant submits that claim 21 has been broadened and now recites that it is only necessary to determine the presence of the antibody, wherein the assay is carried out simply to determine this, without the need for any quantitation. New claim 47 recites, *inter alia*, the method of determining the amount of newly synthesized target antibody. It is possible to determine the amount of antibody, wherein one of ordinary skill in the art simply has to use routine procedures (*e.g.*, serial dilutions such as is carried out in the standard ELISA) and appropriate comparisons with standard control graphs showing the results of detecting known amounts of antibody. This is carried out, for example, in Examples 1 and 2 of the specification as originally filed. Quantitative methods are also clearly referred to throughout the application text. (*See, for example*, pages 5-7, pages 20-21, Tables 3 and 4 and Figure 1). The specification as-filed clearly shows that quantitation can be performed (*e.g.*, by using ELISA).

Applicant respectfully submits that in view of the amendments to claim 21, the rejection of claims 21 under 35 U.S.C. § 112, second paragraph, should be withdrawn.

Claim 34 was objected to as depending from a cancelled claim. Applicant has amended claim 34 thus rendering the rejection moot.

Claim 35 was rejected as under 35 U.S.C. § 112, second paragraph, as having improper antecedent basis. Applicant has amended claim 35 thus rendering the rejection moot.

Claims 38 and 39 are rejected on pages 4-5 of the office action as vague and indefinite because it is allegedly unclear what significant functional cooperative relationship exists between the “one or more antigens” (claim 38) or one or more antibodies (claim 39) recited in the instant claim and the other elements recited in the solid phase binding assay, which requires that they “are contacted with the solid phase” for use in detecting the newly synthesized target antibodies. The Examiner further asks if these antigens (claim 38) or antibodies (claim 39) are intended to provide a label.

Applicant respectfully traverses each of the rejections for at least the following reasons. Applicant submits that there are different methods that may be used for isolating and then detecting the target antibodies. For the isolation step, one way in which binding of the antibody to the solid support may be achieved is through binding to its antigen where the antigen is carried on the solid support. (*See, e.g.,* claim 29). Alternatively the target antibody may bind to an antibody, which is attached to the solid support. (*See, e.g.,* claim 31).

As mentioned on page 14, second paragraph, of the specification as-filed, the detection step may be performed either by binding antibodies to the antibody-antigen complex (or to the antibody-antibody complex) on the solid support, or by binding antigen to the antibody-antibody complex on the solid support. Thus claims 38 and 39 deal with sandwich assays and in claim 38 the following arrangement exists:

[solid support]-[binding partner = antibody to target antibody] - [target antibody] - [antigen to target antibody], and the claim refers to the step of contacting the antigen with the [solid support]-[binding partner = antibody to target antibody] - [target antibody] complex that is already present.

In claim 39 the following two arrangements may exist:

1. [solid support] - [binding partner = antigen to target antibody] - [target antibody] - [antibody to target antibody]; or

2. [solid support]-[binding partner = antibody to target antibody] - [target antibody] - [antibody to target antibody].

Claim 39 recites the step of contacting the antibody (to the target antibody) with the [solid support]-[binding partner = antigen to target antibody] - [target antibody] complex that is already present (as in (1)) or to the [solid support]-[binding partner = antibody to target antibody] - [target antibody] complex that is already present (as in (2)).

Claim 38 depends from claim 31. Claim 31 describes the following configuration:

[solid support]-[binding partner = antibody to target antibody].

Claim 38 thus covers the configuration of claim 31 to which the target antibody has been added:

[solid support]-[binding partner = antibody to target antibody]-[target antibody],

which is contacted with an antigen that recognises the target antibody thus resulting in:

[solid support]-[binding partner = antibody to target antibody] - [target antibody] - [antigen to target antibody].

Claim 39 depends from both of claim 29 and claim 31. Claim 29 corresponds to a configuration, whereby the antigen to the target antibody is carried by the solid support:

[solid support]-[binding partner = antigen to target antibody], and claim 39 when dependent on claim 29 thus covers the configuration of claim 29 to which the target antibody has been added:

[solid support]-[binding partner = antigen to target antibody]-[target antibody],

which is contacted with an antibody that recognizes the target antibody thus resulting in:

[solid support]-[binding partner = antigen to target antibody] - [target antibody] -
[antibody to target antibody].

As discussed above in connection with claim 38, claim 31 corresponds to a configuration, whereby the antibody to the target antibody is carried by the solid support and claim 39 when dependent on claim 31 thus covers the configuration of claim 31 to which the target antibody has been added:

[solid support]-[binding partner = antibody to target antibody]-[target antibody],

which is contacted with an antibody that recognizes the target antibody thus resulting in:

[solid support]-[binding partner = antibody to target antibody] - [target antibody] -
[antibody to target antibody].

Applicant directs the Examiner's attention to page 14, lines 23-25 of the application as-filed and submit that the antigens or antibodies may be labeled to assist detection.

Applicant respectfully submits that in view of the above, the rejection of claims 38 and 39 under 35 U.S.C. § 112, second paragraph, should be withdrawn.

Claim 40 is rejected on page 5 of the office action as allegedly vague and indefinite because it is unclear how the "soluble substrate" is used for the detection step to yield a spectrophotometric signal. The Examiner questions, is the target antibody

labeled with an enzyme for reaction with soluble substrate to hence, produce a spectrophotometric signal.

Applicant submits that claim 40 has been amended to overcome the rejection. In particular, Applicant submits that on page 16, lines 3-19 of the application as-filed, it is set forth that an enzyme-antibody conjugate or an enzyme-antigen conjugate can be used. Applicant points out that the target antibody is the antibody that is to be detected, and this antibody is thus not directly labeled with an enzyme for reaction with a soluble substrate. One of ordinary skill in the art would readily recognize that this would not be possible since only components that are added by the person carrying out the method to the assay can be actively labeled. As such, although added antibody or antigen (as appropriate) can be directly labeled with an enzyme for reaction with a soluble substrate so as to detect the target antibody (via specific interactions between the enzyme-antibody conjugate or the enzyme-antigen conjugate and the target antibody), it is not the target antibody itself that is directly labeled. It is clearly taught page 16, lines 3-19 that the use of the enzyme antibody conjugate or an enzyme antigen conjugate permits estimation of antibody production.

II. The Rejections Under 35 U.S.C. § 103

A. The Rejections

Claim 21, 22, 24-29, 31, and 33-42 are rejected on pages 5-8 of the office action under 35 U.S.C. § 103(a) as allegedly obvious over Choi or Atkinson in view of Cox.

Claim 32 is rejected on pages 8-10 of the office action under 35 U.S.C. § 103(a) as allegedly obvious over Choi or Atkinson in view of Sison.

Applicant respectfully traverses this rejection for the following reasons.

B. The Rejection of Claim 21, 22, 24-29, 31, and 33-42 Over Choi or Atkinson in view of Cox Should be Withdrawn

According to the office action, it would have been obvious to one of ordinary skill in the art at the time of the instant invention to perform the method as taught by Choi or

Atkinson on peripheral blood samples as taught by Cox because Cox provided that lymphocytes used in the method of Choi or Atkinson can be obtained and cultured from peripheral blood samples for use in testing antibody production in response to parenteral influenza vaccination. The office action further alleges that peripheral blood appears to constitute an obvious variation of sample routinely used in the art, upon which lymphocytic cells can be obtained for use in antibody production assays.

Applicant first stresses that the pending claims encompass the detection of “newly synthesized antibodies.” Neither Choi nor Atkinson teaches or suggests methods, which detect newly synthesized antibodies, and the combination of either of these references with Cox fails to remedy this deficiency.

Applicant respectfully submits that the definition of “newly synthesized antibody” can be found on page 4, lines 15-21 of the application as-filed. Indeed, “newly synthesized antibody” is defined as follows:

As used herein, the term “newly synthesized antibody” refers to an antigenically active antibody (i.e. capable of recognizing and binding to the antigen corresponding to the immunogen) which has been produced or synthesized by and within a lymphocyte cell in response to the presence of an immunogen *in vivo* as part of an ongoing immune response. Thus, the antibody is synthesized by a lymphocyte during the course of an immune response triggered by the presentation of an immunogen *in vivo*, i.e. synthesized before and at the time the lymphocyte-containing sample is removed from the subject animal.

See specification at page 4, lines 15-21.

It is important to again emphasize that the claimed assay is specifically directed to the detection of newly synthesized antibodies and that the newly synthesized antibody has been produced by lymphocytes in response to the presence of an immunogen *in vivo* as a part of an ongoing immune response. These antibodies are synthesized before and at the time the lymphocyte containing sample is removed from the subject animal. This is distinct from antibodies that are synthesized *in vitro* during incubation as set out in the

assays described in Choi and Atkinson. Neither Choi nor Atkinson relates to the detection of newly synthesized antibodies as recited by the pending claims.

According to the office action, the claims do not exclude the long incubation times that are taught by the combined teaching of Choi or Atkinson with Cox or Sisson. The fact that the claims refer to detection of “newly synthesized antibodies,” as defined in the instant application, resulting from cell lysis implicitly excludes the use of long incubation times. If long incubation times were to be used, the newly synthesized antibodies would have been secreted from the lymphocytes by the time that the lysis was carried out. Antibodies synthesized *in vitro* during the incubation period would also be detected. As such, it is clear that the claims exclude long incubation times.

Lysis in this context is intended to refer to both freeze thawing based lysis and lysis using chemical means. The specification discloses that “disrupting” is synonymous with “lysing,” and these terms refer to the release of the cell content of the lymphocytes from within the confines of the cell membrane. Secretion does not fall within the scope of this definition.

The Choi and Atkinson assays do not detect newly synthesized antibodies as defined and as claimed as they are antibodies that are synthesized *in vitro*. In particular, the references detect antibody that has been synthesized *in vitro*. Applicant directs the Examiner’s attention to the definition of “newly synthesized antibodies” as defined in the present invention. Each of the references relates to the detection of distinct antibodies to those that are claimed. The crux of the invention relates to the detection of “newly synthesized antibodies.” The combination of references does not disclose or suggest that lymphocytes (*e.g.*, taken from a blood sample) would contain enough antibody to be able to detect the antibody without performing long *in vitro* incubations with antigen. These long incubations allow antibody (*e.g.*, labeled as in Choi, or secreted as in Atkinson) that is synthesized *in vitro* to build up in the culture medium. In contrast, simply lysing cells taken directly from a blood sample would not be expected to yield detectable levels of antibody.

Applicants further point out that the detection of "newly synthesized antibody" is not described in Choi or in Atkinson alone or in combination with Cox for the following reasons. Choi describes a general method by which protein synthesis (in this case biosynthesis of Ig) can be detected. Isolated lymphoid cells are incubated with radioactively labeled leucine. After the requisite length of incubation, the cultures are chilled, centrifuged to separate the cells from the medium and the cells are lysed. The lysate and the supernatant (medium) are then both subjected to TCA precipitation to determine the total amount of radioactive proteins synthesized (*i.e.*, the total amount of proteins synthesized since the label was added, *i.e.*, total amount of all proteins that have been synthesized *in vitro*). A serological assay is also carried out to measure the ratio of labeled Ig.

It is thus clear that the method of Choi detects all proteins that have been synthesized *in vitro* and only proteins that have been synthesized after the addition of the radio-labeled amino acid can be detected. Ig molecules are a subset of these. Newly synthesized antibodies, as defined in the specification text, are thus not detected by the method of Choi. As such even if the teaching of Choi were to be combined with that of Cox, and the method were to be carried out on lymphocytes taken from a blood sample, newly synthesized antibodies would not be detected. Only antibodies that had been synthesized during the *in vitro* incubation would be detected in this way. The combination of Choi and Cox therefore cannot lead to a method falling within the claim scope.

With respect to the Atkinson, Atkinson also does not disclose or suggest how to detect the synthesis of newly synthesized antibodies as defined in the present application text. Atkinson is entirely concerned with devising a method for detecting antibody secretion from cells in suspension during a six hour incubation period *in vitro*. In general, the assays described in Atkinson only measure antibody secretion. There is a single assay in which cells are lysed, but as discussed in more detail below this is a control assay, which was carried out in order to address a specific question and as such there is no motivation to repeat this experiment, and particularly no motivation to repeat

this experiment using lymphocytes taken from a blood sample. This is evident from the paragraph bridging pages 366 and 367, which describes how secreted antibody may be measured in plasma and the paragraph bridging pages 367 and 368, which describes how to modify the ELISA when cell suspensions are used. Furthermore, in the results section it is clear that the assay for *in vitro* antibody production uses cell suspensions. (See, e.g., line 1 of the first paragraph of the Results, Figure 1 and line 1 of the Discussion).

The entire rationale of the Atkinson document is to devise a method for detecting antibody secretion in cell suspensions. For example Figure 1 measures antibody production from spleen/lymph node cell suspensions, and Figure 2 is a control to look at the effect of antibody carry over. The authors also carried out a set of control experiments using puromycin, the results of which are shown in two different Figures, to confirm that synthesis of the secreted antibody occurs *in vitro* (Figure 3) and to check that the puromycin compound has a direct inhibitory effect on the synthesis of Ig molecules. The latter is the only experiment in which any cell lysis is carried out. In order to put the experiments whose results are shown in Figures 3 and 4 into the appropriate context, it is necessary to consider why they were carried out. It is noted in the Results section that puromycin, which causes premature polypeptide termination, was added to cells and, as shown in Figure 3, this eliminates the secretion of antibody during the six hour incubation at 37 °C. The experiment shown in Figure 4 was simply carried out to determine whether the inhibition of antibody secretion as a result of puromycin addition (seen in Figure 3) was due to the direct inhibition of the production of antibody or whether it was due to indirect inhibition i.e. inhibition of a protein that was required for the transport and secretion of intracellular immunoglobulin. Page 369, last 2 lines, confirms that inhibition of synthesis of a transport protein was ruled out by the Figure 4 results. On page 372, lines 7-8 these results are interpreted as indicating that most antibody is secreted in the first 3 hours of culture. The only other reference to the meaning of the Figure 4 results appears in the final three lines of the conclusion. The conclusions that are drawn from this are that "antibody detected is due largely to release of *in vitro* synthesized antibody." Applicant thus submits that it should be appreciated

that the experiments described in Figure 3 and Figure 4 are merely control, investigative experiments, which are carried out by Atkinson to validate their approach to detecting antibody production *in vitro* by measuring antibody secretion.

There is no teaching or suggestion in Atkinson that the measurement of antibody from lysed spleen cells had any wider application than in this control experiment. There is certainly no suggestion that the measurement of antibody from lysed spleen cells could be used for any analytical means, or to replace the method that was in fact itself being tested by Atkinson et al (measurement of secreted antibodies).

Thus, there is no teaching or suggestion to carry out assay in which lymphocytes taken from a blood sample are lysed based on the teaching of Atkinson or Atkinson in combination with Cox.

Indeed, Atkinson's own disclosure goes on to show an experiment (Figure 5) in which antibody production is determined using cell suspensions (2×10^6 cells are added to the top wells, see figure legend). The discussion in Atkinson goes on to confirm the general teaching of the reference relates to antibody secretion (page 371 lines 1-2 of the discussion). The teaching of Atkinson is that antibody secretion can be measured by incubation of cell suspensions under appropriate conditions. There is thus no teaching or suggesting in Atkinson alone or in combination with Cox in the prior art that newly synthesized antibody would be present in cells at a level at which it could be detected.

Applicants respectfully submit that for at least the above reasons, the rejection of claims 21, 22, 24-29, 31, and 33-42 under 35 U.S.C. § 103(a) should be withdrawn.

Applicants further submit that in view of the arguments set forth above with regard to the Cox and Atkinson references for independent claim 21, the rejection of claim 32 (which depends from claim 21) under 35 U.S.C. § 103(a) should also be withdrawn.

With the exception of extension of time fees, no fee is believed due for this submission. However, except for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any necessary fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17,

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which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-0310.

Respectfully submitted,

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